



Research Article

Physicochemical Properties and Antioxidant Potential of Gels from Foam Mat Drying and Ethanolic Extract of Red Spinach (*Amaranthus cruentus*)

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Abstract

Red spinach (*Amaranthus cruentus*), a rich source of antioxidant anthocyanins, is susceptible to light degradation. Foam mat drying (FMD) is a promising technique to preserve these pigments. This study compared the physicochemical properties and antioxidant activity of gels incorporating FMD and ethanolic extracts of *A. cruentus* (EEAC). Both were incorporated into gels at varying concentrations (1%, 3%, and 5%). FMD gels exhibited a darker red color and significantly higher total anthocyanin content ($8.33 \pm 0.25/100$ g) and stronger antioxidant activity (IC_{50} of 35.67 ± 1.87 ppm) compared to EEAC gels ($10.45 \pm 0.15/100$ g and IC_{50} of 47.88 ± 2.45 ppm, respectively). Both gel types had similar pH values (5.32-5.77). Increasing the concentration of either extract affected the viscosity, spreadability, and adhesion of the gels. Importantly, FMD gels displayed significantly higher antioxidant activity (58.75 ± 2.12 to $64.72 \pm 2.01\%$) than EEAC gels (31.75 ± 2.13 to $50.12 \pm 3.01\%$) across all formulations. These findings suggest that FMD-based gels offer a superior delivery system for *A. cruentus* antioxidants, potentially leading to innovative food products with enhanced nutritional value and health benefits.

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INTRODUCTION

Natural plant extracts have gained significant popularity in the cosmetic industry, offering consumers a preference for products derived from natural sources. However, the incorporation of these extracts often presents challenges. One major concern is the inherent instability of many plant-based pigments, which can undergo undesirable color changes, such as darkening, during storage. This phenomenon is typically attributed to the oxidation of bioactive compounds within the extract¹. Various approaches have been explored to enhance the stability of these natural pigments, including encapsulation, lyophilization, and oxygen exclusion². While effective in some cases, these methods may not be readily applicable to all cosmetic formulations, particularly those with a gel-based consistency, and can pose challenges for large-scale industrial production.

Red spinach (*Amaranthus cruentus*) is a rich source of anthocyanins, a class of pigments with potent antioxidant properties^{3,4}. Anthocyanins, naturally occurring pigments responsible for the vibrant colors in many fruits and vegetables, possess potent antioxidant properties with potential health benefits⁵. These pigments are responsible for the vibrant red color of the leaves. However, anthocyanins are susceptible to degradation due to environmental factors such as high temperature, intense light exposure, and changes in pH^{6,7}. In alkaline conditions, for instance, anthocyanins undergo structural changes and form

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colorless compounds⁸. Despite these challenges, *A. cruentus* extracts have demonstrated significant antioxidant activity in various studies. Moilati *et al.*⁹ reported potent antioxidant activity of *A. cruentus* ethanol extract with an IC₅₀ value of 2.82 ppm. Similarly, Ni'am *et al.*¹⁰ observed potent antioxidant activity in *A. cruentus* extract sheet masks, with an IC₅₀ value of 68.55 ppm. These findings highlight the potential of *A. cruentus* extracts as a valuable source of natural antioxidants for various applications.

Extraction is a widely employed technique to isolate bioactive compounds from natural sources. However, extracted natural products often exhibit rapid color degradation, resulting in a darkening of the extract¹¹. To mitigate this issue, various drying methods have been explored. Foam mat drying (FMD) has emerged as a promising technique for preserving the color and quality of natural products¹². In FMD, maltodextrin acts as a foaming agent, enabling the formation of a foam matrix that is subsequently dried at low temperatures under vacuum conditions¹³. This gentle drying process minimizes the risk of thermal degradation and oxidation of heat-sensitive bioactive compounds¹⁴. Furthermore, FMD produces a dried product with desirable characteristics, such as vibrant color, appealing aroma, and pleasant taste, often closely resembling the fresh material¹⁵. The FMD process also offers significant advantages in terms of drying time, typically completing within 24 hours. The resulting dried product is typically a fine, crystalline powder with low moisture content and intense color¹⁶.

Encapsulation technologies have emerged as promising approaches for improving the stability and bioavailability of these bioactive compounds. While previous research¹⁷ has explored the microencapsulation of anthocyanins for food preservation using hydrogel matrices, their application in topical formulations has been limited. This study aims to investigate the physical characteristics and antioxidant activity of FMD containing *A. cruentus* extract encapsulated within a hydrogel matrix. By comparing the properties of these FMD with those of a *A. cruentus* extract gel, this research seeks to evaluate the potential of this encapsulation technique for developing stable and effective topical delivery systems for anthocyanin-rich extracts.

MATERIALS AND METHODS

Materials

Fresh *A. cruentus* leaves were obtained from the Bandungan area in Semarang Regency, Indonesia. The plant species was authenticated by the Herbarium of the Semarang College of Pharmaceutical Sciences (STIFAR), with a certificate number of 026/EL-AFM/II/2023. The gel base formulation consisted of carbopol 940, Tween 80, Span 80, sorbitol, liquid paraffin, triethanolamine (TEA), methylparaben, propylparaben, and distilled water, all of pharmaceutical grade and purchased from Multi Kimia Raya Chemical. Dimethyl sulfoxide (DMSO), used as a solvent, was of analytical grade and obtained from Sigma Aldrich through PT Kairos.

Methods

Extraction

Fresh *A. cruentus* leaves (1 kg) were thoroughly washed with distilled water to remove any adhering soil and debris. The washed leaves were then sorted to remove any discolored or damaged parts. Subsequently, the leaves were dried in an oven at 40°C for 8 hours until they reached a constant weight. The dried leaves were then ground into a fine powder using a blender. Maceration was employed for the extraction process. Briefly, 100 g of the powdered plant material was macerated with 500 mL of 96% ethanol for 24 hours at room temperature. This process was repeated three times (3x24 hours) with fresh ethanol each time. After each maceration step, the extract was filtered through Whatman No. 1 filter paper. The combined filtrates were then concentrated using a rotary evaporator at 50°C under reduced pressure to obtain a thick extract. The yield of the extract was calculated gravimetrically. Finally, the obtained thick ethanolic extracts of *A. cruentus* (EEAC) was incorporated into a gel base according to the formulation described by previous research¹⁸.

Foam mat drying of *A. cruentus*

Fresh *A. cruentus* leaves were meticulously washed to remove any adhering dirt and potential pests. Subsequently, the leaves were air-dried under ambient conditions to eliminate excess moisture. A 1:1 (w/v) ratio of fresh leaves to distilled water was employed for the preparation of a leaf slurry. This slurry was then homogenized using a high-speed blender. To enhance foam formation, 6% (v/v) Tween 80 was incorporated into the homogenate and the mixture was vigorously mixed

for 8 minutes using a laboratory mixer. Subsequently, 15% (w/w) maltodextrin was added to the mixture and stirred for an additional 3 minutes. The resulting mixture was carefully spread onto aluminum foil trays lined with 1 mm thick aluminum foil. These trays were then placed in a tray dryer maintained at a constant temperature of 40°C for 60 minutes. The drying process was continued until a constant weight was achieved, indicating complete moisture removal. Finally, the dried *A. cruentus* leaf FMD was pulverized and sieved through a No. 60 mesh sieve to obtain a fine powder¹⁹.

Formulation of EEAC and FMD gels

Carbopol 940 (1%) was dispersed in 10 mL of distilled water heated to 80°C in a glass beaker with constant stirring for 1 hour. The dispersion was then transferred to a mortar and neutralized with triethanolamine dropwise under continuous stirring until a transparent gel (Mixture A) was formed. Separately, hydroxypropyl methylcellulose (HPMC) (0.5%) was also dispersed in 10 mL of distilled water at 80°C for 1 hour and then transferred to a mortar. The HPMC dispersion was stirred until a transparent gel (Mixture B) was obtained. Methylparaben (0.15%) was dissolved in propylene glycol (Mixture C). Subsequently, Mixtures A and B were sequentially added to Mixture C with continuous stirring until a homogenous gel base was formed. The EEAC (1% w/w) was dissolved in 5 mL of 96% ethanol and then incorporated into the gel base with gentle stirring. For the FMD formulations, the *A. cruentus* FMD (1% w/w) was dissolved in propylene glycol and added to the gel base. Finally, distilled water was added to the mixtures to achieve the desired final volume and ensure homogeneity^{2,20}. **Table I** summarizes the compositions of the EEAC and FMD gel formulations.

Table I. Formulation of EEAC and FMD gels.

Materials	Concentration (g)					
	F1	F2	F3	F4	F5	F6
<i>Amaranthus cruentus</i> FMD	1	3	5	-	-	-
<i>Amaranthus cruentus</i> EEAC	-	-	-	1	3	5
Carbopol 940	1	1	1	1	1	1
HPMC	0.5	0.5	0.5	0.5	0.5	0.5
Propylene glycol	10	10	10	10	10	10
Methylparaben	0.18	0.18	0.18	0.18	0.18	0.18
Triethanolamine	3	3	3	3	3	3
Distilled water	Up to 100					

Determination of anthocyanin levels

To assess the stability of EEAC and FMD under simulated gastric (pH 1.0) and intestinal (pH 4.5) conditions, solutions were prepared by dissolving a known amount of EEAC or FMD in respective buffer solutions. The ratio of EEAC/FMD to buffer was maintained at 1:5 (v/v). The prepared solutions were incubated at room temperature for 15 minutes. Subsequently, the absorbance of each solution was measured at the maximum wavelength of 512 nm using a spectrophotometer. To correct for any background interference, a second measurement was taken at 700 nm²¹.

Physical properties of gel

Physical characterization of the gels was conducted to assess their suitability for topical application. Organoleptic properties, including color, odor, and texture, were evaluated through direct visual observation. Homogeneity was assessed by visually inspecting the gel for any visible phase separation or sedimentation after gentle shaking. pH was measured using a calibrated pH meter. Viscosity was determined using a viscometer, and spreadability as well as adhesion was evaluated by measuring the time taken (minutes) for a defined volume of gel to spread over a specified area (cm) on a glass slide²².

Antioxidant activity test

The antioxidant activity of the EEAC, FMD, and the developed gel formulations was evaluated using the 2,2-diphenyl-1-picrylhydrazyl (DPPH) free radical scavenging assay. For the EEAC and FMD, a series of concentrations (50, 100, 150, 200, and 250 ppm) were prepared. One milliliter of each sample was mixed with 2 mL of 0.1 mM DPPH solution in a test tube, and the volume was adjusted to 5 mL with ethanol. The mixture was incubated for 30 minutes at room temperature in the dark. Subsequently, the absorbance of the reaction mixture was measured at 516.12 nm using a spectrophotometer. The percentage of DPPH radical scavenging activity was calculated, and the IC₅₀ values, representing the concentration of the sample required to inhibit 50% of DPPH radicals, were determined²³.

For the gel samples, 1 g of each gel formulation was dissolved in 10 mL of 96% ethanol. Three milliliters of this solution was then mixed with 2 mL of 0.1 mM DPPH solution, shaken vigorously, and incubated for 30 minutes at room temperature in the dark. The absorbance of the reaction mixture was measured at 516.12 nm using a spectrophotometer. The percentage of DPPH radical scavenging activity of the gel formulations was subsequently calculated.

Data analysis

To assess the impact of the incorporated extract on the physicochemical properties of the gels, a two-way ANOVA was employed to analyze data on total anthocyanin content, pH, viscosity, stickiness, spreadability, and percentage inhibition. This statistical method allowed for the evaluation of the effects of both the gel formula (FMD vs. EEAC) on these parameters. Organoleptic properties, including appearance, odor, and texture, were evaluated and described descriptively.

RESULTS AND DISCUSSION

The extraction of *A. cruentus* yielded a 38.86% thick EEAC, characterized by a dark brown color and a distinctive *A. cruentus* aroma. In contrast, the FMD exhibited a 92.43% yield and presented as a dark red powder with a characteristic *A. cruentus* aroma. **Figure 1** visually depicts the physical appearance of both the EEAC and FMD. The significant difference in yield between the two methods can be attributed to the foaming process involved in FMD preparation, which necessitates the addition of a foaming agent to the *A. cruentus* powder. Conversely, the traditional extraction method, involving solvent evaporation, leads to a substantial loss of mass due to the removal of the solvent.

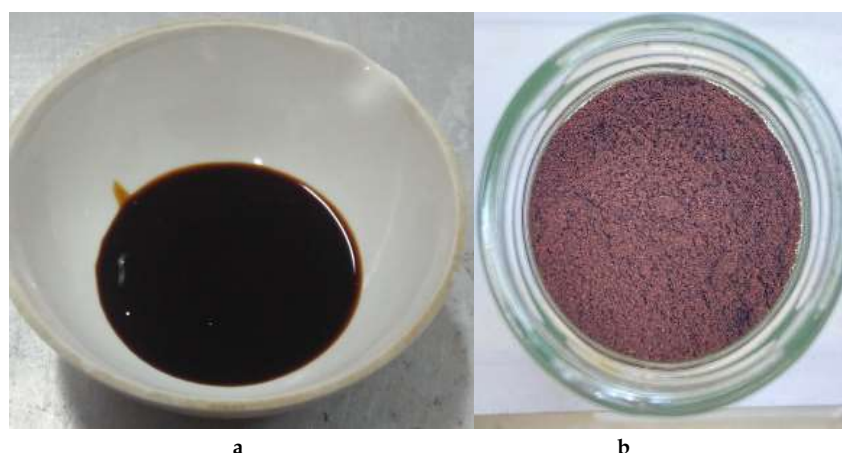


Figure 1. Appearance of (a) EEAC and (b) FMD of *A. cruentus*.

Table II presents the total anthocyanin content of EEAC and FMD. Statistical analysis revealed a significant difference ($p < 0.05$) in anthocyanin content between the two samples, with FMD exhibiting significantly higher levels than the ethanolic extract. This discrepancy can be attributed to the inherent limitations of the conventional extraction process. Prolonged extraction times and elevated temperatures can lead to the degradation of heat-sensitive anthocyanins. Studies have shown that high temperatures accelerate the degradation of these pigments²⁴. Moreover, the extraction process itself can contribute to the oxidation and degradation of flavonoid compounds, including anthocyanins²⁵. Drying methods involving temperatures exceeding 60°C can further exacerbate this issue, leading to the oxidation of anthocyanins and a noticeable darkening of the sample²⁶.

In contrast, the FMD effectively preserves the labile anthocyanin pigments. By rapidly removing water through sublimation, FMD minimizes thermal degradation and oxidative processes, resulting in higher retention of these bioactive compounds. This finding aligns with previous research highlighting the advantages of FMD for preserving the stability and bioactivity of heat-sensitive compounds¹³. Furthermore, the FMD process offers several advantages over conventional drying methods, including lower operating costs, reduced drying time, and lower energy consumption²⁷. These factors make FMD a more suitable and sustainable approach for preserving the bioactive compounds of *A. cruentus* for various applications.

Table II. Anthocyanin levels in EEAC and FMD.

Sample (n=3)	Yield (%)	Anthocyanin levels \pm SD (per 100 g)	IC ₅₀ \pm SD (ppm)
<i>Amaranthus cruentus</i> EEAC	38.86 \pm 4.20	8.33 \pm 0.25	47.88 \pm 2.45
<i>Amaranthus cruentus</i> FMD	92.43 \pm 2.21	10.45 \pm 0.15	35.67 \pm 1.87

The visual characteristics of the gels formulated with EEAC and FMD are depicted in [Figure 2](#). The EEAC gel exhibited a dark brown color, a characteristic *A. cruentus* odor, and a sticky consistency. In contrast, the FMD gel displayed a dark red color, a distinctive *A. cruentus* scent, and a rapid film-forming property upon application to the skin.

**Figure 2.** Gels made from EEAC (a-c) and FMD (d-f). (a) F1, (b) F2, (c) F3, (d) F4, (e) F5, and (f) F6.

A comprehensive overview of the physical characteristics of both gel formulations is presented in [Table III](#). The pH of the formulated gels was evaluated to assess their stability and potential for topical application. No significant differences in pH were observed between the gels prepared with EEAC and those prepared with FMD. This finding suggests that the drying method did not significantly alter the acidic nature of the extract. Furthermore, an increase in the concentration of *A. cruentus* extract resulted in a decrease in the pH of the gels. This observation is consistent with the acidic nature of anthocyanins, the primary bioactive compounds in *A. cruentus*, which are known to lower the pH of solutions^{16,28}.

The present study investigated the influence of increasing concentrations of EEAC and FMD on the viscosity of developed hydrogels. A significant increase in viscosity was observed with increasing concentrations of both EEAC and FMD. This finding aligns with previous research, which demonstrated that an increase in the concentration of natural polymers and bioactive compounds generally leads to an increase in the viscosity of formulations²⁹. Notably, within each formula (varying EEAC and FMD concentrations), a significant difference in viscosity was observed, indicating that the concentration of the active substances directly influenced this rheological property. However, statistical analysis revealed no significant difference in viscosity between gels containing the same concentration of active substances, regardless of whether the active substance was EEAC or FMD. This suggests that the viscosity of the hydrogel is primarily influenced by the overall concentration of the active ingredient, rather than the specific type of active substance.

The spreadability of the EEAC and FMD gels was evaluated at the same concentration. No significant differences in spreadability were observed between the two formulations. This finding aligns with previous research demonstrating that increasing the concentration of active substances within a gel matrix can lead to an increase in viscosity, consequently reducing its spreadability³⁰. This inverse relationship between viscosity and spreadability is well-established in the literature³¹.

The adhesion properties of the EEAC and FMD gels were evaluated. Notably, no significant differences in adhesion were observed between gels containing the same concentration of active ingredients, nor were any variations found between different formulations. These findings align with previous research conducted by Rahmawati *et al.*³², which reported no discernible effect of increasing the concentration of active substances on gel adhesion. However, Puspita *et al.*³³ demonstrated that exceeding an active substance concentration of 10% can indeed influence adhesion properties. The present study suggests that within the concentration ranges investigated, the active ingredients in EEAC and FMD gels did not significantly impact their adhesive properties.

The IC₅₀ values of the ethanolic extract of EEAC and FMD gels exhibited significant differences ($p < 0.05$). The FMD gel demonstrated higher IC₅₀ values compared to the EEAC gel, indicating a lower antioxidant activity. These findings were consistent with the observed gel inhibition percentages. Both EEAC and FMD gels demonstrated a dose-dependent increase in antioxidant activity, aligning with previous research that higher concentrations of bioactive compounds generally correlate with increased antioxidant activity³⁴.

The enhanced antioxidant activity of the FMD gel can be attributed to the milder processing conditions employed during drying. The FMD process operates at lower temperatures compared to the conventional extraction method³⁴, which is crucial for preserving the stability of heat-sensitive compounds such as anthocyanins. Previous studies have reported that anthocyanins are susceptible to degradation at temperatures exceeding 50°C^{35,36}. Furthermore, the shorter processing time of the FMD process (3 hours) compared to the 8-hour extraction process may have minimized the potential for degradation of bioactive compounds.

Table III. Physical characterization of EEAC and FMD gels.

Parameters (n = 3)	Formula					
	F1	F2	F3	F4	F5	F6
pH	5.77 ± 0.68	5.65 ± 0.72	5.45 ± 0.88	5.68 ± 0.65	5.54 ± 0.71	5.32 ± 0.84
Viscosity (cps)	98.45 ± 2.33 ^a	115 ± 4.18 ^a	122 ± 8.75 ^a	95.21 ± 3.41 ^b	120.25 ± 8.14 ^b	134.15 ± 5.18 ^b
Spreadability (cm)	5.63 ± 0.11 ^a	4.77 ± 0.14 ^a	3.81 ± 0.12 ^a	5.56 ± 0.13 ^b	4.68 ± 0.1 ^b	3.78 ± 0.11 ^b
Adhesion (minutes)	2.28 ± 0.55 ^a	2.33 ± 0.61 ^a	2.41 ± 0.54 ^a	2.31 ± 0.47 ^b	2.45 ± 0.63 ^b	2.32 ± 0.48 ^b
% inhibition	31.75 ± 2.13 ^{ac}	48.66 ± 3.15 ^{ac}	50.12 ± 3.01 ^{ac}	58.75 ± 2.12 ^{bc}	61.19 ± 2.35 ^{bc}	64.72 ± 2.01 ^{bc}

Note: ^a: significantly different between each EEAC formula; ^b: significantly different between each PMK formula; ^c: significantly different between EEAC compared to FMD at the same concentration.

Foam mat drying demonstrated significant potential in enhancing the stability of anthocyanin pigments within *Amaranthus cruentus* extract. The presence of maltodextrin in the foaming process likely acted as a protective agent, encapsulating the pigments and minimizing their degradation during drying³⁷. This is supported by the observation that FMD-dried *A. cruentus* exhibited superior color retention compared to conventionally dried samples. Furthermore, the relatively short drying time and low-temperature processing conditions inherent to FMD contribute to its efficiency and minimize potential oxidative damage to the pigments³⁸. These findings align with previous studies that have successfully employed FMD to preserve the color and bioactive compounds of various plant materials, particularly those containing sensitive phytochemicals^{39,40}.

The enhanced stability of anthocyanins in FMD-dried *A. cruentus* was reflected in the improved antioxidant activity observed in this study. This finding corroborates the results of Farid *et al.*⁴¹, who reported significant antioxidant activity and flavonoid content in FMD-dried plant materials. The optimal maltodextrin concentration for maximizing anthocyanin retention in this study (10%) aligns with previous findings⁴². Moreover, the low-temperature drying conditions employed in FMD (below 50°C) are known to effectively preserve the phytochemical content of plant extracts. The observed enhancement in antioxidant activity in the *A. cruentus* gel formulated with FMD-dried extract further supports the efficacy of this drying method. The foam matrix likely provides a protective environment that minimizes the degradation of anthocyanins and other phenolic compounds during processing and storage⁴³.

CONCLUSION

This study demonstrated that FMD successfully preserved the bioactive properties of *A. cruentus* leaves. The FMD powder exhibited enhanced antioxidant activity compared to the conventionally dried extract, as evidenced by a lower IC₅₀ value in the DPPH assay. Furthermore, the incorporation of FMD powder into gel formulations resulted in improved antioxidant activity compared to gels containing the conventionally dried extract. This finding suggests that FMD effectively preserves the bioactive compounds, particularly anthocyanins, responsible for the antioxidant activity. Importantly, the FMD process also maintained the color and overall physical characteristics of the gels, indicating its potential for producing stable and visually appealing formulations. These findings highlight the potential of FMD as a promising technique for preserving the bioactive properties and enhancing the utilization of *A. cruentus* leaves in various food and pharmaceutical applications.

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DATA AVAILABILITY

None.

CONFLICT OF INTEREST

The authors declare no conflicts of interest related to this study.

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